Evolutionary and Acclimation-Induced Variation in the Thermal Limits of Heart Function in Congeneric Marine Snails (Genus *Tegula*): Implications for Vertical Zonation

EMILY STENSENG, CAREN E. BRABY*, AND GEORGE N. SOMERO

Hopkins Marine Station, Department of Biological Sciences, Stanford University, Pacific Grove, California 93950-3094

Abstract. We analyzed the thermal limits of heart function for congeneric species of the marine snail *Tegula* that have different patterns of vertical zonation. *T. funebralis* is found in the low to mid-intertidal zone, and *T. brunnea* and *T. montereyi* live in the low-intertidal or subtidally. As indices of thermal limits of heart function, we used the temperature at which heart rate initially decreased rapidly during heating (the Arrhenius break temperature, or ABT) and the temperature at which heart ceased to beat with either heating or cooling (the flatline temperature, or FLT_{hot} or FLT_{cold}, respectively). These three indices provide an estimate of the thermal range within which *Tegula* heart function is maintained. For field-acclimatized specimens, the thermal range of the high-intertidal *T. funebralis* was greater than those of its two lower-occurring congeners (higher ABT, higher FLT_{hot}, lower FLT_{cold}). We also demonstrated the effects of constant thermal acclimation on the heart rate response to heat stress. Acclimation to 14 °C and 22 °C resulted in increases in ABT and FLT_{hot}, with the largest changes in *T. brunnea* and *T. montereyi*. Although *T. funebralis* is more heat tolerant and eurythermal than its two lower-occurring congeners, it can encounter field body temperatures that exceed ABT, indicating that *T. funebralis* faces a larger threat from heat stress, *in situ*. These findings are consistent with recent studies on other taxa of marine invertebrates that have shown, somewhat paradoxically, that warm-adapted, eurythermal intertidal species may be more impacted by global warming than congeneric subtidal species that are less heat tolerant.

Introduction

Physiological adaptations to temperature in ectothermic species are pervasive and have long been regarded as important in establishing biogeographic patterning along latitudinal thermal gradients (Bullock, 1955). Studies of many taxa of marine invertebrates have documented that physiological adaptations to temperature also play key roles in establishing vertical zonation along the subtidal to intertidal gradient (reviewed in Newell, 1979; Somero, 2002). In these analyses of how temperature-adaptive physiological variation contributes to biogeographic and vertical patterning, congeneric species are especially powerful study systems (Stillman and Somero, 1996; Stillman, 2002, 2003; Tomanek, 2002). These closely related species allow the effects of temperature to be discerned because confounding influences due to phylogeny are absent (Stillman and Somero, 2000). Along the gradient of subtidal to intertidal habitats, a wide range of phyla have congeneric species with different vertical zonation ranges, which allows the linkages between adaptation and zonation to be compared.

Studies of congeneric species of the turban snail *Tegula* from central California (Tomanek and Somero, 1999) have shown that a low-to-mid-intertidal species, *T. funebralis* (A. Adams 1855), is more tolerant of high temperatures than two congeners, *T. brunnea* (Philippi, 1848), and *T. montereyi* (Kiener, 1850), that occur exclusively in the very

low intertidal zone or subtidally (hereafter referred to as subtidal species). In an effort to understand the mechanistic bases of these differences in thermal tolerance, we examined the abilities of these three congeners of *Tegula* to sustain heart function at high and low temperatures. We determined the temperature at which heart function initially showed a sharp decrease with rising temperature, the Arrhenius break temperature (ABT) (Dahlhoff *et al.*, 1991; Stillman and Somero, 1996), and the temperature at which heart rate fell to zero, the flatline temperature (FLT_{hot}). The effects of cold stress on heart rate were compared by recording the temperature at which the heart ceased beating during chilling (FLT_{cold}). To determine the relative acclimatory capacities (physiological plasticity) of the three congeners, animals were acclimatized to 14 °C and 22 °C. In agreement with recent studies of porcelain crabs (genus *Petrolisthes*) from subtidal and intertidal habitats (Stillman and Somero, 1996, 2000; Stillman, 2003), the low- to mid-intertidal species *T. funebralis* is significantly more heat tolerant and eurythermal than the subtidal species. Nonetheless, *T. funebralis* faces the largest threat from heat stress *in situ* because its body temperature may routinely reach levels at which heart function is impaired and because it is less able to acclimate to higher temperatures than its more cold-adapted, low-occurring congeners. Thus, somewhat paradoxically, species with relatively high abilities to tolerate heat may be the most threatened by global warming.

Materials and Methods

Study organisms and acclimation protocols

Tegula funebralis has a broad biogeographic distribution in the eastern Pacific. It occurs in the low- to mid-intertidal zone from Vancouver Island, British Columbia, Canada (48°25'N) to central Baja California, Mexico (28°0'N) (Abbott and Haderlie, 1980; Riedman *et al.*, 1981). *Tegula brunnea* occurs in the subtidal to low-intertidal zones of the eastern Pacific Ocean from Cape Arago, Oregon (43°C25'N) to the Santa Barbara Channel Islands, California (34°17'N) (Abbott and Haderlie, 1980; Riedman *et al.*, 1981; Watanabe, 1984). *Tegula montereyi* occurs almost exclusively in the subtidal zone from Sonoma County, California (38°17'N) to the Santa Barbara Channel Islands (Abbott and Haderlie, 1980; Riedman *et al.*, 1981; Watanabe, 1984). Specimens used in these studies were collected in May and June 2004 at Hopkins Marine Station of Stanford University in Pacific Grove, California (36°36'N, 121°54'W). All specimens were adults of medium to large size (20–25 mm basal diameter). Specimens designated as "field acclimatized" were held in recirculating aquaria containing ambient seawater (13–14 °C) for 24 h prior to experimentation.

Acclimation experiments were conducted in two recirculating seawater aquaria with water temperatures set to 14°C and 22 °C (±0.2 °C). Fifteen snails of each species were acclimated for 15–19 days. Animals were fed fresh kelp (*Macrocystis pyrifera*) every 3 days during the acclimation period. Water levels in the tanks were kept high enough to prevent emersion.

Measurements of heart rate

A hand-held drill was used to make two small holes (diameter about 1 mm) in the shell of each snail, adjacent to the pericardial space. Ceramic-coated copper electrodes were inserted into these holes, placed as close as possible to the heart, and secured with cyanoacrylate adhesive. The impedance between the two electrodes, which changed as a function of distance as the heart beat, was converted to a voltage signal using impedance converters (model 2991, UFI, Morro Bay, CA) and recorded using a PowerLab data acquisition system (ADI Instruments, Castle Hill, Australia). To prevent the snails from emerging from their shells during experimentation, the outer lip of the shell was glued to a clean glass microscope slide. Corks were then glued to the top of the shell, and the animals were suspended, by metal clamps attached to the corks, in a temperature-controlled water bath containing filtered and aerated seawater. The temperature of the water bath was controlled by a programmable, computer-controlled Lauda refrigerated water bath whose temperature could be ramped up or down at the desired rate. Six animals were run concurrently in each experiment. A gelatin-filled snail shell in which a thermocouple was implanted was placed in the experimental water bath to monitor the rate of change in "body" temperature during heating or cooling. The heating or cooling of gelatin-filled shells occurs at the same rate as in intact snails (Tomanek and Somero, 1999). Thermal equilibration was essentially instantaneous; that is, there was no measurable difference between the temperatures of the immersion water bath and the gelatin-filled snail shell (data not shown).

For heat-stress experiments, the water bath temperature was held constant at 13 °C for 1 h and then increased by 1 °C every 15 min (see Fig. 1A) a rate that is environmentally realistic (Tomanek and Somero, 1999). Heart rate was measured every 7 min during the experiment. After reaching 40 °C, or once all specimens' hearts had failed, water temperature was decreased rapidly to 13 °C to assess recovery of cardiac function.

For cold-stress experiments, 6 specimens of each species were maintained at 13 °C for 1 h, and then the water temperature was decreased at a rate of 1 °C per 10 min, down to a temperature near 0 °C. After cessation of heart beat, the water temperature was quickly returned to 13 °C to assess recovery of cardiac function.

Following the heat- and cold-stress experiments, snails were removed from the experimental water bath and placed in an aquarium containing 13–15 °C seawater. Survivorship was recorded over a 72-h period.

In addition to temperature ramps, control runs were performed to ensure that the stress of experimentation (drilling, electrode placement and gluing) did not contribute to heart failure or mortality. In the control experiments, heart rates were monitored for 7 h at 13 °C, the ambient seawater temperature during the time of these studies. Survival of the control snails was monitored for a week after the experiments. All snails survived (data not shown).

Data analysis

Heart rates were expressed as beats per minute (bpm). Arrhenius plots (ln bpm *versus* reciprocal temperature (K)) were generated, and the Arrhenius break temperature (ABT)—defined as the temperature at which the Arrhenius plot exhibited a sharp discontinuity in slope (*i.e.*, a rapid decrease in bpm once a certain temperature was reached)—was calculated as described by Dahlhoff *et al.* (1991) and Stillman and Somero (1996). ABT was determined, using a standard spreadsheet program by drawing two best-fit regression lines, one on either side of the putative inflection point on the Arrhenius plot (Fig. 1B). The intersection of these two regression lines was used to determine the ABT in degrees Celsius.

Flatline temperatures (FLT_{hot} or FLT_{cold}) were recorded as the temperatures at which the heart beat ceased, at either high or low temperatures.

The effects of temperature and species on ABT and FLT were evaluated using analysis of variance (ANOVA). For analyzing data from field-acclimatized snails, we used one-factor ANOVA (with species as the only factor), while for the data from acclimated specimens we used two-factor ANOVA (with species and acclimation temperature as the two factors). Significant ANOVA results were followed by *post hoc* comparisons to discern the differences among species (Tukey test, $\alpha = 0.05$). Data are given as means ± standard error.

Results

Habitat and body temperatures

During the animal collection period (May–June 2004), sea surface temperatures at our study site (Hopkins Marine Station) were near 13 °C and ambient air temperatures seldom exceeded 20 °C. Thus, the subtidal species, *Tegula brunnea* and *T. montereyi*, were probably exposed to a near-constant temperature of 13 °C prior to experimentation. *T. funebralis*, in contrast, undergoes substantial changes in body temperature in concert with alternating periods of immersion and emersion during the tidal cycle. During a several week period in March and April of 1996, Tomanek and Somero (1999) recorded body temperatures at the same site as ours; they showed that body temperatures were as high as 33 °C for *T. funebralis*, and that tempera-

tures in the range of 27–33 °C were common. In April 2000, Tomanek and Sanford (2003) recorded body temperatures as high as 34.5 °C for *T. funebralis*, consistent with the earlier measurements. During the 1996 study, body temperatures of *T. brunnea* only rarely reached 24 °C. *T. montereyi*, which has a lower vertical position than *T. brunnea*, would not experience body temperatures in excess of ambient water temperatures unless an unusual emersion event occurred. Although our study site on Monterey Bay is near the middle of the distribution ranges of the species, our conclusions about the thermal conditions of the subtidal species during immersion would apply over their full biogeographic ranges, because both species are restricted to cool, mid-latitude habitats in which surface seawater temperature rarely reaches 20 °C (National Climatic Data Center: http://www.ncdc.noaa.gov/oa/ncdc.html.)

Field-acclimatized snails

We initially measured the effects of temperature on the heart rate of field-acclimatized snails. The response of all specimens was qualitatively similar: beats per minute (bpm) initially rose with increasing experimental temperature and then abruptly decreased as a species-specific high temperature, the ABT, was reached (Fig. 1A). ABT values (mean ± SE) for the three congeners were 31.1 ± 0.7 °C for *T. funebralis*, 25.0 ± 0.5 °C for *T. brunnea*, and 24.2 ± 0.7 °C for *T. montereyi* (Fig. 2). ABT values differed significantly by species (one-factor ANOVA, $P < 0.0001$), with *T. funebralis* exhibiting a higher ABT than the two subtidal species; ABTs for the latter two species are not significantly different from each other. In heating experiments, FLT_{hot} values (temperatures at which heart rate fell to zero) for the three congeners were 39.4 ± 0.2 °C for *T. funebralis*, 32.4 ± 0.2 °C for *T. brunnea*, and 33.1 ± 0.1 °C for *T. montereyi* (Fig. 2). As with ABT, FLT_{hot} values differed significantly by species (one-factor ANOVA, $P < 0.0001$), with *T. funebralis* exhibiting a higher FLT_{hot} than the two subtidal species.

When heat-stressed snails taken to the FLT_{hot} were returned to 13 °C, they regained cardiac activity (Fig. 1A). However, within 24–48 h after return to 13 °C, 92% of the *T. funebralis* specimens and 100% of the *T. brunnea* and *T. montereyi* died.

As specimens were cooled from 13 °C down to about 0 °C, heart beat gradually decreased in rate and eventually ceased. No distinct ABT could be determined from the data, but FLTs could be discerned and were significantly different among species (one-factor ANOVA, $P = 0.0005$). *Tegula funebralis* had a significantly greater tolerance of low temperatures, as well as high temperatures; its FLT_{cold} was 2.1 ± 0.2 °C, compared to values of 3.5 ± 0.3 °C and 4.8 ± 0.5 °C for *T. brunnea* and *T. montereyi*, respectively, which were not significantly different from each other. All speci-
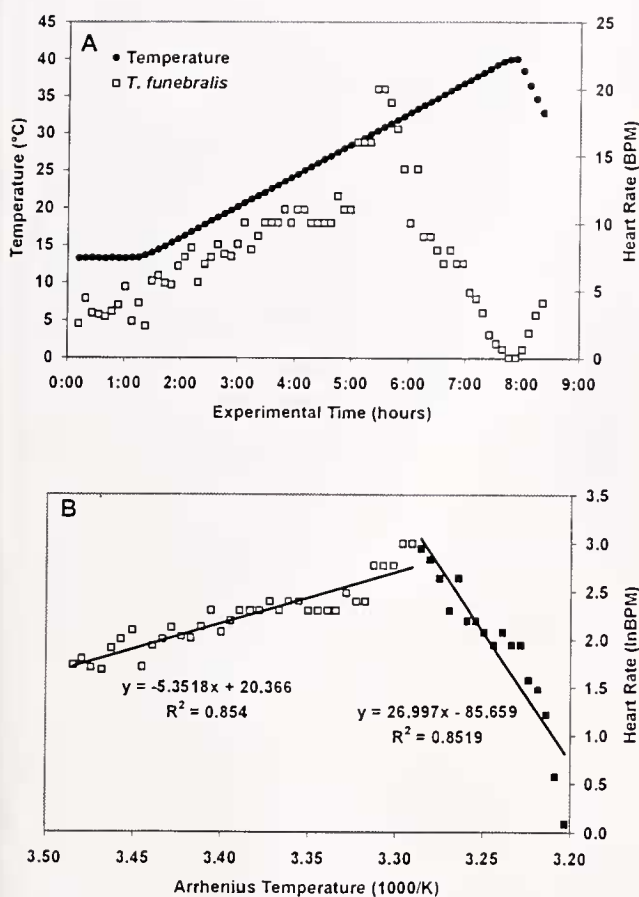
Figure 1. Effects of increasing measurement temperature on heart rate of field-acclimatized *Tegula funebralis*. (A) Typical response of heart rate to increasing temperature. (B) Arrhenius plot of the same data, indicating the method for calculating Arrhenius break temperature (ABT) (see Materials and Methods).

mens subjected to cold stress recovered, and no mortality was observed during the subsequent 3-day holding period.

At a common measurement temperature, the heart rate of *T. funebralis* was significantly higher than those of the other two species. At 13 °C, heart rates were as follows: *T. funebralis*, 6.8 ± 1.3 bpm; *T. brunnea*: 2.0 ± 0.3 bpm; and *T. montereyi*, 3.6 ± 0.4 bpm.

Laboratory-acclimated snails

To examine phenotypic plasticity in ABT and FLT_{hot}, we acclimated the three species to two temperatures, 14 °C and 22 °C. The former temperature corresponds closely to the average water temperature and thus the average body temperatures of the two lower-occurring species) at the season of study (spring); the latter temperature reflects the highest temperatures recorded for *T. brunnea* (Tomanek and Somero, 1999). Both acclimation temperature and species contributed significantly to the patterns of ABT and FLT_{hot} (two-factor ANOVA, $P < 0.0001$ for both ABT and FLT).
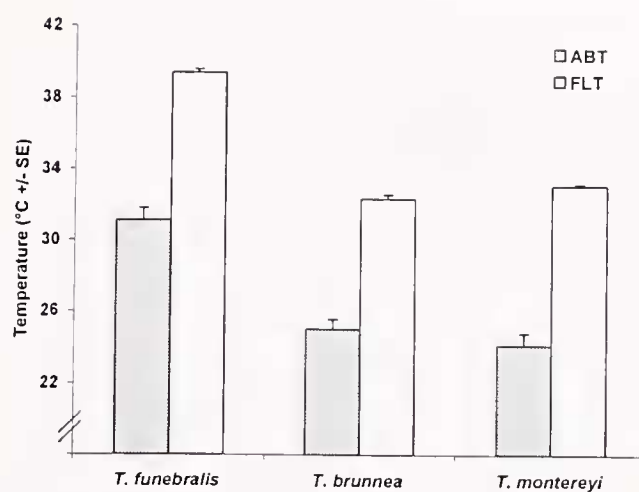

Figure 2. Arrhenius break temperature (ABT) and flatline temperature (FLT_{hot}) values for three congeners of field-acclimatized *Tegula* subjected to heating. Numbers of specimens: *T. funebralis* (13), *T. brunnea* (11), and *T. montereyi* (10). Error bars represent standard errors of the mean.

In all species, the higher acclimation temperature increased both ABT and FLT_{hot}. However, acclimation had stronger effects on the ABT values of the two lower-occurring species, which increased their ABT values by 4 °C (*T. montereyi*) or 6.6 °C (*T. brunnea*), than on that of *T. funebralis*, which increased its ABT by only 1.6 °C (Table 1). Only very small increases in FLT_{hot} were noted (Table 1) but all species were significantly different from each other.

Discussion

The differences in thermal effects on cardiac function among these three congeners of *Tegula* provide insights into

Table 1

Values for Arrhenius break temperature (ABT) and flatline temperature (FLT) (°C) for congeners of Tegula *acclimated to temperatures of 14 °C or 22 °C for 15–19 d or taken directly from the field*

Variable	Acclimation type	*T. funebralis*	*T. brunnea*	*T. montereyi*
Heat stress				
ABT	14 °C	28.5 ± 0.5 °C	20.2 ± 0.8 °C	21.7 ± 0.8 °C
	22 °C	30.1 ± 0.7 °C	26.8 ± 0.7 °C	25.7 ± 0.6 °C
	Field	31.0 ± 0.7 °C	25.0 ± 0.5 °C	24.2 ± 0.7 °C
FLT	14 °C	39.8 ± 0.2 °C	31.7 ± 0.4 °C	33.6 ± 0.3 °C
	22 °C	40.6 ± 0.2 °C	32.8 ± 0.3 °C	34.1 ± 0.2 °C
	Field	39.4 ± 0.2 °C	32.4 ± 0.2 °C	33.1 ± 0.1 °C
Cold stress				
FLT	Field	2.1 ± 0.2 °C	3.5 ± 0.3 °C	4.8 ± 0.5 °C

Number of individuals in each group: acclimated to temperatures of 14 °C or 22 °C (*T. funebralis* = 6; *T. brunnea* = 5; *T. montereyi* = 6) or taken directly from the field (heat stress — *T. funebralis* = 13, *T. brunnea* = 11, *T. montereyi* = 10; cold stress — *T. funebralis* = 6, *T. brunnea* = 6, *T. montereyi* = 6).

the determinants of vertical zonation and the differential effects that climate change may have on these species. We measured two variables to determine thermal effects: the Arrhenius break temperature (ABT), or the temperature at which heart rate decreases rapidly as temperature rises; and the flatline temperature (FLT_{hot} or FLT_{cold}), or the temperature at which heart beat ceases, at either high or low temperatures. The observation that the cardiac function of *T. brunnea* and *T. montereyi* becomes impaired at body temperatures near 24–25 °C, the ABTs for both species, and ceases near 32–33 °C, the FLT_{hot} values of the two species (Fig. 2), shows that neither species is adapted to function at the body temperatures that are common for *T. funebralis* during emersion (Tomanek and Somero, 1999, 2000; Tomanek and Sanford, 2003). Even though the ABTs and, to a lesser extent, the FLT_{hot} values of the two subtidal species were increased during acclimation to high temperature, *T. brunnea* and *T. montereyi* still lack the ability to withstand temperatures that *T. funebralis* encounters during emersion. Although all individuals recovered cardiac function immediately when temperature was reduced to values below the FLT_{hot} (Fig. 1A), no individuals of *T. brunnea* and *T. montereyi* survived during subsequent incubation at 13 °C. Thus, even though cessation of heart activity is not immediately lethal, thermal damage done during exposure to temperatures as high as the FLT_{hot} eventually proved lethal to both subtidal species. Studies of thermal effects on protein synthesis and expression of heat-shock proteins showed that these processes, too, were fully inhibited at temperatures near 33 °C in *T. brunnea* and *T. montereyi* (Tomanek and Somero, 1999). For *T. funebralis*, protein synthesis and production of heat-shock proteins continued to temperatures near 38 °C. Interspecific differences in thermal tolerance were also observed in field experiments in which specimens of *T. brunnea* were caged and transplanted to intertidal sites at which *T. funebralis* is common. During the one month of exposure to intertidal conditions within the cages, no mortality was observed for *T. funebralis*, but 8.5% of the *T. brunnea* specimens died (Tomanek and Sanford, 2003).

The impairment of cardiac function noted in these experiments, which involved only submerged specimens, may underestimate the stress encountered in the field, especially by the intertidal species *T. funebralis*. During emersion, it is possible that stress from desiccation or restricted gas exchange could exacerbate the effects resulting from temperature. For example, if respiratory stress during emersion reduces oxygen supply to the heart, lower ABT and FLT_{hot} values than those reported here may result. We are unaware of any studies of marine invertebrates that have measured thermal limits of heart function in air *versus* water. Santini *et al.* (1999) reported that heart rate in the intertidal limpet *Patella caerulea* was the same in emersed and submerged specimens at temperatures up to about 25 °C, but thermal limits were not determined. A multifactorial analysis of the

effects of thermal, respiratory, and desiccation stress could provide a more refined characterization of the limits of cardiac function in intertidal species.

Another possible determinant of cardiac thermal sensitivity that merits further study is the role that variation in temperature, as opposed to average temperature, plays in setting ABT values. Here we show that ABT values for the field-acclimatized specimens of *T. brunnea* and *T. montereyi* (which experience average sea surface temperatures of about 13° C) were more similar to those of the 22 °C-acclimated snails than to those of the 14 °C-acclimated individuals. This suggests that laboratory acclimation studies done at a constant temperature (and thus simulating the average field temperature) may yield data that differ substantially from those obtained from specimens that are field-acclimatized to a similar average, but more variable, temperature. Although we did not determine the ranges of body temperature experienced by the field-acclimatized specimens of the subtidal species, perhaps even short periods at elevated temperatures were sufficient to elicit an adaptive response, that is, an increase in the ABT to values higher than expected on the basis of average temperature.

The increases in ABT noted following acclimation to 14 °C and 22 °C were not paired with equivalent changes in FLT_{hot}. The basis of this discrepancy in the phenotypic plasticity of the two traits is not known, but it might reflect different mechanistic bases for ABT and FLT_{hot}. ABT effects are commonly attributed to alterations in the biophysical properties of membranes (Hochachka and Somero, 2002). Because the composition of membranes can be adaptively altered during thermal acclimation through processes termed homeoviscous and homeophasic adaptation, it is likely that the shifts in ABT reflect such alterations. Dahlhoff and Somero (1993) found correlated changes in the ABT of mitochondrial respiration and membrane biophysical state (fluidity) during thermal acclimation of abalone (genus *Haliotis*). FLT_{hot} values may, in contrast to ABT, reflect thermal effects on cellular constituents such as proteins that are unable to alter their temperature sensitivities during acclimation.

The differences in heat tolerance of cardiac function found among congeners of *Tegula* reveal that interspecific differences in vulnerability to heat death *in situ* exist. The most heat-tolerant and eurythermal species studied, *T. funebralis*, is most threatened by extremes of high temperature. The ABT for cardiac function is slightly below the upper body temperatures encountered during emersion on hot days at a site near the middle of the species' latitudinal distribution range (Tomanek and Somero, 1999). Thus, unlike the two subtidal species, which are unlikely to experience seawater temperatures as high as their ABTs throughout their biogeographic ranges (National Climatic Data Center: http://www.ncdc.noaa.gov/oa/ncdc.html), *T. funebralis* occasionally encounters these temperatures during em-

ersion. Moreover, *T. funebralis* has a lower ability to increase its ABT during acclimation to warmer temperatures. Thus, even though it is more tolerant to heat, *T. funebralis* seems more likely than its subtidal congeners to encounter damaging, if not lethal, temperatures *in situ*. Its limited ability to acclimate to increased temperatures suggests that *T. funebralis* will be more imperiled by global warming than are its lower-occurring congeners. And, as indicated above, if respiratory and desiccation stresses compound the effects of stress from high temperature, *T. funebralis* may be even more threatened by warming than is suggested by these studies done with submerged specimens.

These same interspecific trends were observed in subtidal and low-to-mid-intertidal porcelain crabs (Stillman and Somero, 1996, 2000; Stillman, 2003). The highest occurring temperate porcelain crab had ABTs for heart function that essentially coincided with the highest body temperatures it encounters in the field, and it had a lower ability to increase ABT during warm acclimation. Another parallel with porcelain crabs is found in the interspecific differences in cold tolerance. The more heat-tolerant intertidal species in each genus also sustain heart function at lower body temperatures than do subtidal species (Table 1); that is, they are significantly more eurythermal than subtidal species. The differences in cold tolerance of intertidal and subtidal species may also contribute to vertical zonation, because mid-latitude winter temperatures for emersed intertidal organisms may fall below 0 °C. The mechanistic basis of heart stoppage at low temperatures is not clear. However, the effects of low temperature seem consistent with a gradual reduction in metabolic rate (*i.e.*, in the supply of ATP to support cardiac activity) that eventually leads to cessation of heart beat.

One additional difference between *T. funebralis* and the two subtidal congeners merits noting: the significantly higher rate of heart beat in the intertidal species. Other marine molluscs exhibit a significant positive correlation between heart rate and rate of oxygen consumption (metabolism)(see Santini *et al.*, 1999, for references). Our findings suggest, then, that under identical thermal conditions, *T. funebralis* may have a higher rate of metabolism than its subtidal congeners. Because *T. funebralis* grows significantly more slowly than either *T. brunnea* or *T. montereyi* (Frank, 1965; Watanabe, 1982; Somero, 2002), its higher rate of energy turnover may reflect increased costs of repairing thermal damage. Supporting this hypothesis is evidence from studies of the expression of heat-shock proteins in field-acclimatized individuals of these three species (Tomanek and Somero, 2002). Compared to *T. brunnea* and *T. montereyi*, *T. funebralis* had higher standing stocks of the heat-inducible isoform of heat-shock protein 70 (Hsp72) as well as a higher ratio of Hsp72 to Hsp74, a constitutively expressed chaperone. Similarly, field-acclimatized specimens of *T. funebralis* also had higher standing stocks of the

transcription factor, heat-shock factor-1, which modulates expression of heat-shock genes.

In summary, the differences in the responses of heart activity to increases or decreases in body temperature found in three congeneric turban snails having different vertical distributions at a common latitude illustrate the importance of physiological adaptation in establishing vertical zonation in marine species. As in the case of porcelain crabs, the species that lives highest in the intertidal zone and is subject to prolonged periods of emersion is more tolerant of high and low temperatures than are the low-intertidal or subtidal species. However, the most eurythermal and heat-tolerant congeners are also the most threatened by heat stress because the upper value of their temperatures are close to the temperatures that stop their hearts, and because they have limited abilities to extend the heat tolerance of heart function during acclimation to higher temperatures.

Acknowledgments

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